IN THE CLAIMS

Please amend the claims as follows:

Cancel Claims 1 and 2.

B2

3. (Once amended) The transgenic non-human female mammal [An animal] of claim [2] 33, wherein said [promoter] additional DNA segment required for expression is selected from the group consisting of a whey acid protein promoter, a casein promoter, a ß-lactoglobulin promoter, and an α-lactalbumin promoter.

Cancel claims 4-10

11. (Once amended) The transgenic non-human female mammal [An animal] of claim [1] 33, wherein said mammal [animal] is selected from the group consisting of a rodent, rabbit, [cat, dog,] pig, sheep, goat, and cattle [cow or horse].

B3

12. (Once amended) The transgenic non-human female mammal [An animal] of claim [1] of 33 wherein said fibrinogen [protein] comprises a modified fibrinogen [molecule] wherein said modification does not adversely affect the biological activity of the fibrinogen.

13. (Once amended) The transgenic non-human female mammal [An animal] of claim [1] 33, wherein said fibrinogen [protein] comprises a fusion protein comprising a human fibrinogen and [with] a non-fibrinogen protein which facilitates purification of the fibrinogen from said milk.

Cancel claims 14 and 15.

B4

16. (Once amended) The method [A process] of claim [15] 32, wherein said [promoter] additional DNA segment required for expression is selected from the group consisting of a whey acid protein promoter, a casein promoter, a ß-lactoglobulin promoter, and an α-lactalbumin promoter.

B

Cancel claim 17-19.

B5 20. (Once amended) The method [A process] of claim [15] 32, wherein said fibrinogen protein comprises a fusion protein comprising a human fibrinogen and [with] a non-fibrinogen protein which facilitates purification of the fibrinogen from said milk.

Cancel claim 21.

B6 22. (Once amended) The method [A process] of claim [15] 32, wherein said mammal [animal] is selected from the group consisting of a rodent, rabbit, [cat, dog,] pig, sheep, goat, and cattle [cow or horse].

Cancel claim 23-28.

B7 29. (Once amended) The process [A process] of claim [15] 32, wherein said fibrinogen comprises a modified fibrinogen [molecule] wherein said modification does not adversely affect the biological activity of the fibrinogen.

B8 31. (Once amended) A prokaryotic cell transformed by a DNA [plasmid according to claim 30] comprising a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Aα chain, a second DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Bβ chain, or a third DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen γ chain, and further wherein each chain is operably linked to additional DNA segments required for its expression in said mammal.

Please add the following new claims:

B9 34. The method of claim 32, wherein the bred male and female offspring that are transgenic are used to expand the transgenic herd.

B

35. The method of claim 32, wherein said human fibrinogen comprises biologically active natural variants of human fibrinogen that are converted to fibrin upon reaction with human thrombin.

36. The transgenic non-human mammal of claim 33, wherein said human fibrinogen comprises biologically active natural variants of human fibrinogen that are converted to fibrin upon reaction with human thrombin.

37. Isolated cells obtained from the transgenic non-human mammal of claim 33, wherein said cells contain organelles of said cells and said DNA segments are stably integrated into the genome of said cells, and wherein said cells produce biologically active human fibrinogen which is converted to fibrin upon reaction with human thrombin..

38. A method for producing biologically active human fibrinogen that is converted to fibrin upon reaction with human thrombin comprising:

providing a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Aα chain, a second DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Bβ chain, and a third DNA γ chain, wherein each of said first, second, and third segments is operably linked segment encoding a secretion signal operably linked to a heterologous fibrinogen to additional DNA segments required for its expression in said mammal;

introducing said DNA segments by transgenic cells or organelles into a non-human mammalian species heterologous to the species of origin of said fibrinogen chains to produce a transgenic animal;

breeding said transgenic animals to produce progeny that express said first, second and third DNA segments encoded by said DNA segments and which produce milk containing biologically active human fibrinogen that is converted to fibrin upon reaction with human thrombin;

collecting said milk from said progeny; and

recovering the biologically active human fibrinogen from the milk, wherein said fibrinogen is converted to fibrin upon reaction with thrombin.

B9 Cont.

B

39. The method of claim 38, wherein the bred male and female offspring that are transgenic are used to expand the transgenic herd.

40. A transgenic non-human mammal that produces recoverable amounts of biologically active human fibrinogen in the milk of said mammal, wherein said fibrinogen is converted to fibrin upon reaction with at least one protease, wherein said mammal comprises:

a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Aα chain,

a second DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Bß chain, and

a third DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen γ chain, and

further wherein each chain is operably linked to additional DNA segments required for its expression in said mammal.

41. A transgenic non-human mammal that produces biologically active transgenic human fibrinogen wherein said fibrinogen is converted to clinically useful fibrin upon reaction with at least one protease.

B9 cont.

42. Isolated cells obtained from the transgenic non-human mammal of claim 40, wherein said cells contain organelles of said cells and said DNA segments are stably integrated into the genome of said cells, and wherein said cells produce biologically active human fibrinogen which is converted to fibrin upon reaction with at least one protease.

43. Purified biologically active transgenic human fibrinogen produced by the mammal of claim 33, wherein said fibrinogen is converted to fibrin by thrombin.

44. Purified biologically active transgenic human fibrinogen produced by the mammal of claim 40, wherein said fibrinogen is converted to fibrin by at least one protease.

B

45. Clinically useful fibrin made from transgenic human fibrinogen produced by the mammal of claim 40, wherein the fibrin is cross-linked by an enzyme having transglutaminase activity.

46. Clinically useful fibrin made from transgenic human fibrinogen produced by the mammal of claim 33, wherein the fibrin is cross-linked by an enzyme having transglutaminase activity.

47. A transgenic non-human mammal that produces recoverable amounts of biologically active human fibrinogen in a non-milk body fluid of said mammal, wherein said fibrinogen is converted to fibrin upon reaction with human thrombin, wherein said mammal comprises:

a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Aα chain,

a second DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Bß chain, and

a third DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen γ chain, and

further wherein each chain is operably linked to additional DNA segments required for its expression in said mammal.

B9 cont.

48. The transgenic non-human mammal of claim 47, wherein said non-milk body fluid is selected from the group consisting of blood, a fraction of blood and urine.

49. The transgenic non-human mammal of claim 47, wherein said human fibrinogen comprises a modified fibrinogen or natural variant of said fibrinogen wherein said modification or variant does not adversely affect the biological activity of the fibrinogen.

50. A transgenic non-human mammal that produces recoverable amounts of biologically active non-human mammalian fibrinogen in a non-milk body fluid of said mammal, wherein said fibrinogen is converted to fibrin upon reaction with non-human mammalian thrombin, wherein said mammal comprises:

B

a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Aα chain,

a second DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Bβ chain, and

a third DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen γ chain, and

further wherein each chain is operably linked to additional DNA segments required for its expression in said mammal.

51. The transgenic non-human mammal of claim 50, wherein said body fluid is selected from the group consisting of milk, blood, a fraction of blood and urine.

52. The transgenic non-human mammal of claim 50, wherein said non-human mammalian fibrinogen is selected from the group consisting of rodent, rabbit, cat, dog, pig, sheep, goat, cow and horse fibrinogen.

53. The transgenic non-human mammal of claim 50, wherein said non-human mammalian fibrinogen comprises a modified fibrinogen or natural variant of said fibrinogen, wherein said modification or variant does not adversely affect the biological activity of the fibrinogen.

B9 Cont.

54. A transgenic non-human mammal containing at least one stably integrated DNA sequence encoding heterologous Aα, Bβ, and γ fibrinogen subunit chains in a germ line cell, wherein said germ line cell confers the ability to said mammal to transfer said DNA sequence to offspring of said mammal.

55. The transgenic non-human mammal of claim 54, wherein said mammal is a female.

56. The transgenic non-human mammal of claim 54, wherein said mammal is male..

B

57. A method for producing biologically active human mammalian fibrinogen that is converted to fibrin upon reaction with human thrombin comprising:

providing a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Aα chain, a second DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Bß chain, and a third DNA γ chain, wherein each of said first, second, and third segments is operably linked segment encoding a secretion signal operably linked to a heterologous fibrinogen to additional DNA segments required for its expression in said mammal;

introducing said DNA segments by transgenic cells or organelles into a non-human mammalian species heterologous to the species of origin of said fibrinogen chains to produce a transgenic animal;

breeding said transgenic animals to produce progeny that express said first, second and third DNA segments and produce a non-milk body fluid containing biologically active human fibrinogen that is converted to fibrin upon reaction with human thrombin encoded by said DNA segments;

collecting said non-milk body fluid from said progeny; and

recovering the biologically active mammalian fibrinogen that is converted to fibrin upon reaction with mammalian thrombin from the no-milk body fluid.

B9 cont

58. The method of claim 57, wherein said fibrinogen comprises a modified fibrinogen or natural variant of said fibrinogen wherein said modification or variant does not adversely affect the biological activity of the fibrinogen..

59. The transgenic non-human mammal of claim 57, wherein said non-milk body fluid is selected from the group consisting of blood, a fraction of blood and urine.

60. A method for producing biologically active non-human mammalian fibrinogen that is converted to fibrin upon reaction with non-human mammalian thrombin comprising:

providing a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen Aα chain, a second DNA segment encoding a

B

secretion signal operably linked to a heterologous fibrinogen Bβ chain, and a third DNA γ chain, wherein each of said first, second, and third segments is operably linked segment encoding a secretion signal operably linked to a heterologous fibrinogen to additional DNA segments required for its expression in said mammal;

introducing said DNA segments by transgenic cells or organelles into a non-human mammalian species heterologous to the species of origin of said fibrinogen chains to produce a transgenic animal;

breeding said transgenic animals to produce progeny that express said first, second and third DNA segments and produce a non-milk body fluid containing biologically active human fibrinogen that is converted to fibrin upon reaction with human thrombin encoded by said DNA segments;

collecting said non-milk body fluid from said progeny; and

recovering the biologically active mammalian fibrinogen that is converted to fibrin upon reaction with mammalian thrombin from the no-milk body fluid.

B9 Cont.

61. The method of claim 60, wherein said body fluid is selected from the group consisting of milk, blood, a fraction of blood and urine.

62. The method of claim 61, wherein said non-human mammalian fibrinogen selected from the group consisting of rodent, rabbit, cat, dog, pig, sheep, goat, cow and horse fibrinogen.

63. The method of claim 61, wherein said fibrinogen comprises a modified fibrinogen or natural variant of said fibrinogen wherein said modification or variant does not adversely affect the biological activity of the fibrinogen.

REMARKS

Claim 3, 11-13, 16, 20, 22, 29, and 31 have been amended. Claims 1, 2, 4-10, 14, 15, 17-19, 21, 23-28, and 30 have been canceled and replaced by new claims 34-63. Claims

B

32 and 33 were added in the response to a request to copy claims. Thus, with the entry of the following amendments, claims 3, 11-13, 16, 20, 22, 29, and 31-63 are active in this application. Support for claims is in the original claims as filed and in the specification. Particularly, the claims directed to the transgenic cells and organelles are supported by the specification on page 8, lines 15-23 which discuss the introduction and integration of DNA into germ line cells. Additionally, beginning on page 7, line 24 to page 8, line 16, which includes the book by Hogan et al., standard methods of transferring DNA, such as organelle transfer or nuclear transfer, are discussed.

Support for claims directed to clinically useful fibrinogen is found in the Examples which show the production of fibrinogen that is proteolytically converted to fibrin by thrombin. It is well known that clinically useful fibrin is formed from the proteolytic degradation of fibrinogen into fibrin by thrombin which is a serine protease. For example, other sources of serine proteases may come from milk, which is known to contain a number of proteases, including thrombin-like proteases that can degrade fibrinogen to fibrin. Milk is also known to contain transglutaminases that can cross-link fibrin to make it more stable. It is well known that milk and other natural products could be sources of proteases and transglutaminases that would be useful to make clinically useful fibrin. Page 19, lines 12-23, support that milk is known to contain a number of proteases, including serine proteases, such as thrombin that degrade fibrinogen into fibrin. It is also known that transglutaminases cross link fibrin to make it more stable. Beginning on page 3, line 24 to page 4, line 2, support is provided for using the fibrinogen of the present invention in "glue", which is know to be cross-linked in some applications. On page 3, line 13 to line 23, Roy et al. is referenced which describes degrading fibrinogen to fibrin with Factor XIII, which is a transglutaminase. Additionally, the Examples provide support to show recoverable amounts of fibrinogen are produced and Example 8 with reference to Fig. 9 show that fibrinogen is converted to fibrin with thrombin and Fig. 11 shows that fibrin is cross-linked due to endogenous transglutaminase activity. In regard to the claims directed to natural variants of fibrinogen, page 1, lines 9-24 discuss that fibrinogen may have congenital abnormalities due to structurally or functionally different molecules. No new matter has been added.

B

1. Priority

The Examiner requests that the application make a specific reference to the earlier filed patent application. The specification has been amended to contain this information.

2. Rejections under 35 U.S.C. § 112, First Paragraph

2.1 Claims 1-11, 15-25, and 28

Claims 1-11, 15-25 and 28 have been rejected because the specification only provides an enabling disclosure for the production of biologically active recombinant unmodified heterologous fibrinogen wherein each subunit of fibrinogen is from the same species, and does not provide an enabling disclosure for the preparation of modified fibrinogen comprising heterologous subunits derived from different species.

Applicants respectfully disagree with the Examiner's position that the specification is only enabled for fibrinogen subunit chain from the same species. The application is intended to encompass non-human mammalian fibrinogen as set forth in original claim 24 as well as human fibrinogen. It is Applicants' position that when the DNA encoding sequences for each of the three fibrinogen subunits from any species are known, then it is well within the skill of the artisan to introduce the these DNA sequences into animals to produce transgenic animals which have stably integrated the DNA sequences into the genome. By following the methods of the present invention, a skilled person can introduced the three fibrinogen subunits from different species into animals. The specification discloses the methods for isolating the body fluid containing the heterologous fibrinogen produced by the transgenic animals, and detecting and quantifying the transgenically produced fibrinogen. The specification on pages 20-22 and the examples disclose numerous methods for determining the biological activity of produced fibrinogen. The Examiner contends that there is no guidance provided as to how a functional fibrinogen molecule comprising subunits from different species. But all that is required by a skilled person is that he carry out the disclosed methods and test for biologically active fibrinogen. No other guidance is required and no undue experimentation is necessary.

Similarly, the Examiner states that modifications and fusion proteins of the transgenically produced fibrinogen are not enabled because there is no disclosure regarding

B

wheat such fusion proteins, mutants, and derivatives would comprise, what activity they would have and how one would prepare them. Again as above, one the DNA sequences are known the DNA can be manipulated and changed as disclosed on page 10, lines 3-28, using well known techniques to introduce site mutagenesis to alter post-translational modifications, such as eliminate glycosylation sites. Any modification to any one or all of the fibrinogen subunits can be assessed by determining the presence of fibrinogen and its biological activity. Therefore, by following the present disclosure, a skilled person can modify the DNA sequences, introduce these sequences into a host, and then assess the fibrinogen produced by the transgenic animals using methods disclosed in the present invention. The Examiner contends that the complexity of the fibrinogen molecule would make it difficult for modified or fusion protein chains to assemble correctly. But as discussed above, it is not necessary to know with certainty the outcome of each change to the fibrinogen molecule. All that is necessary is that the biological activity of the fibrinogen can be tested using disclosed techniques. The present application provides such techniques. Therefore, it is Applicant's position that the specification provides an enabling disclosure for the scope of the claims.

The Examiner additionally contends that the specification fails to disclose the production of fibrinogen in transgenic animals in any tissue than mammary glands and in any other body fluid than milk, and has determined that it would require undue experimentation to do so. In response, Applicants respectfully direct the Examiner's attention to the analogous art where other promoters and other hosts have been used in the transgenic expression of proteins. For instance, the paragraph bridging pages 131-132 and Table 1 of *Transfusion Medicine Reviews*, Vol. X, No. 2:131 (1996) shows the different tissues and body fluids of a number of different host organisms in which various blood proteins are expressed. The text on page 131, second column, recites that

> "Since the mid-1980's, expression of blood proteins has been directed to specific organs like the liver, or to cells like erythrocytes and lymphocytes. The liver-specific promoters of human α_1 AT apollipoptotins (Apo), FIX, and serum anyloid P component genes (SAP) or the erythrocyte-specific globin gene locus, to name a few, have been used to express the respective genes in transgenic mice."

Tables 2 and 3 of the publication show the expression of numerous proteins in the milk of different hosts with different regulatory regions. Additionally, publications by

B

Swanson *et al.* (1992) and Sharma *et al.* Show production of functional human hemoglobin in erythrocytes in blood. Mikkelsen *et al.* demonstrates salivary gland specific expression and secretion of the C-terminal peptide of human Factor VIII into saliva. Massoud *et al.* demonstrates the expression of α1-antitrypsin I the blood of transgenic rabbits and Ruther *et al.* expresses the same protein in liver and macarophages in transgenic mice. Thus, it is well within the skill of the artisan to produce proteins in many different tissues and body fluids of transgenic organisms using the appropriate regulatory sequences which will direct the protein to the tissue in which it is to be expressed. It would only require trial and error experimentation to select appropriate DNA constructs, inject the constructs into the animals and test for expression of correctly assembled fibrinogen in the body fluids. Undue experimentation is not required for such selection. It is requested that these rejections be withdrawn.

2.2 Claims 12-14 , 26, 27, and 29-31

The Examiner rejects claims 12-14, 26, 27, and 29-31 as not being enabled for the reasons set forth above. These claims are directed to modified or fusion proteins of fibrinogen or fibrinogen that is secreted into blood or urine. It is Applicant's position that these claims are enabled for the reasons set forth above

In regard to claim 30, this claim has been canceled. Claim 31 has been amended and is an independent claim. It is requested that these rejections be withdrawn.

3. Rejections under 35 U.S.C. § 112, Second Paragraph

Claim 1-31 are rejected as being indefinite for failing to distinctly claim the invention. Claim 30 has been canceled. Claims 1 and 15 have been canceled and replaced with claims in which "physiologically functional" has been replaced by "biologically active". It is requested that these rejections be withdrawn.

B

4. Rejections under 35 U.S.C. § 103

Claims 1-31 are rejected as being obvious over either Meade *et al.*, (A1) or Rosen *et al.*, (A14) either in view of Clark *et al.* (A4), Wyngaarden *et al.*(A21), Chung *et al.* (A7), Chung *et al.* (A8), and Rixon *et al.* (A9).

The Examiner prefaces his rejection by stating that as a result of the indefiniteness of language "physiologically functional" that the combination of the references is applicable to the claimed animals and methods of making fibrinogen to the extent that the artisan would have been motivated to have prepared fibrinogen molecules that do not have to have any particular activity requirement or limitation. As indicated above, the language "physiologically functional" has been replaced by language suggested by the Examiner, which is "biologically active". All of the claims now require that recoverable amounts of biologically active fibrinogen be produced.

The Examiner has combined a large number of references to reject the present invention and used impermissible hindsight to reject the claims of the present invention. The Examiner states that because Meade and Rosen disclose preparing proteins with therapeutic value in transgenic animals and because fibrinogen was a known therapeutically valuable protein, it would have been obvious to arrive at the present invention. There is simply so suggestion to combine the cited references. It is requested that this rejection be withdrawn.

CONCLUSION

It is believed that applicants have overcome the rejections set forth by the Examiner. Therefore, Applicants believe that the present application is now in condition for allowance, and respectfully request favorable consideration to that effect. Should the Examiner have any issues which he would like to discuss , the Examiner is requested to contact the undersigned attorney.

Respectfully submitted,

Jayme A. Huleatt

Jayme A. Huleatt
Reg. No. 34,485

August 15, 1997
Date

FOLEY & LARDNER
3000 K Street, N.W., Suite 500
P.O. Box 25696
Washington, D.C. 20007-8696
(202) 672-5300

B